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I hereby certify that this paper is being deposited this date with the U.S. Postal Service in first class mail addressed to Commissioner of Patents and Trademarks, Washington, D.C. 20231.

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Date
March 4, 2002

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SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

We have submitted an Information Disclosure Statement on January 8, 2002, along with the application identified above. One of the reference articles submitted, "Die Bedeutung genetischer Faktoren bei der Enstehung des Herzinfarkt," was not in the English language. We submit herewith a translation of that article.

The U.S. application was filed on January 8, 2002, and no fee is due. However, the Patent Office is authorized to charge any fee or credit any overpayment to our deposit account

No. 03-3125. A copy of this sheet is enclosed.

In the absence of more pertinent prior art, the application is believed to be in condition for allowance; favorable action is respectfully solicited.

Respectfully submitted,
COOPER & DUNHAM LLP

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The importance of genetic factors in the development of myocardial infarction

As late as the middle of last century the sudden death of a forty-year-old due to myocardial infarction had to be accepted as an inexplicable and unalterable stroke of fate by his family and friends. In the past decades, however, scientists were increasingly able to identify and even influence the risk factors responsible for such an event.

Pathological changes in the coronary vessels leading to a narrowing are frequent. The sudden occlusion of the artery that provides a particular part of the heart with oxygen-rich blood provokes the death of that part of the heart muscle, i.e. myocardial infarction. The consequences of such pathological changes in the blood vessels are the principal causes of death in industrialized countries. It is less known that the narrowing of coronary and other, bigger, oxygen-carrying vessels, i.e. atherosclerosis, can even affect younger, productive members of society.

Risk factors accelerating the development of atherosclerosis

In the past decades long-term epidemiological studies identified numerous factors that accelerate the development of atherosclerosis. One of the best known of these studies is the so-called "Framingham Heart Study". So far, more than 5000 inhabitants of the North American city of Framingham, Massachusetts, have been medically examined every two years with regard to the development of atherosclerotic changes affecting the blood vessels in dependence of numerous quantifiable factors over a period of four decades.

Figure 1 presents important risk factors leading to an accelerated development of atherosclerosis that have been identified on the basis of such studies. Environmental factors like cigarette smoking, the quality (fat composition, salt content, etc.) and quantity of food, the extent of body exercise, strain or, of late, the infestation of the coronary vessels by microbes are of major interest in the investigation of heart and circulatory diseases. By modification of individual environmental factors and aspects of behaviour positive effects on these risk factors can be achieved. A dietary change, e.g., can lower the plasma concentration of the low-density lipoprotein (LDL) cholesterol that accelerates the development of atherosclerosis. A rise in the high-density lipoprotein (HDL) cholesterol that prevents atherosclerosis can be achieved by increased body exercise and other measures.

Nevertheless, even if environmental factors and aspects of behaviour that increase the risk of atherosclerosis are avoided, already younger adults can develop distinct atherosclerotic changes or even fall victim to a heart attack. In such cases hereditary factors play a decisive role. As far as disorders due to a gene defect are concerned, i.e. a defect in the part of the gene encoding a specific endogenous protein, the above mentioned environmental factors are less important. Here, the effects of the defective gene often outweigh by far a possible modification of the risk factor by environmental influences. For example, in the case of defects in the genes that play a major role in the cholesterol metabolism, often the cholesterol content of the blood cannot be normalized by dietary changes. Defects in the genes, so-called mutations, that, due to the synthesis of a defective protein, increase the risk, influence one or several of these risk factors so strongly that typically before the age of 55 cardiovascular complications occur. The cases are referred to as early-onset or premature atherosclerosis.

Identification of individuals at risk in families where several members are affected by myocardial infarctions

If several members of a family are affected by premature atherosclerosis, straightforward family screening and genetic analysis can help to identify the underlying cause. Specific fragments of the genetic information corresponding to parts of genes are analyzed and compared among the members of a family. The gene defect, if known, can also be screened for directly. By means of these so-called molecular genetic tests an increased risk can be excluded in the family members not affected by the gene defect, and unjustified fears can be dispelled. On the other hand, individuals affected by the gene defect can be made aware of the problem. Today, effective treatment is available for many of the risk factors mentioned in Figure 1; in the case of increased cholesterol levels in the blood, e.g. with cholesterol-lowering drugs. This therapy has the potential to reduce the risk of myocardial infarction even if atherosclerotic changes in the coronary vessels already exist. This was impressively confirmed by the so-called "4S Study" (Scandinavian Simvastatin Survival Study) that investigated the effect of statin treatment in a total of 4444 individuals with elevated cholesterol concentrations and already manifest heart disease. In many cases, the molecular genetic testing of individuals at risk

and their family members can uncover the underlying mechanisms. With regard to those disorders that lead to myocardial infarctions already in younger adults – in extreme cases already in childhood, scientific research focuses on the investigation of the genetic disposition to detect gene defects. In recent years various genes have been discovered that play key roles in the development of the above mentioned risk factors. Several genetic disorders have been demonstrated to lead to premature atherosclerosis by influencing a risk factor. In Figure 2 (gene chart) some of the afore mentioned risk factors (Figure 1) are matched with the respective disorders and the already known genes responsible for these disorders.

The importance of genetic factors in the development of atherosclerosis: cholesterol as an example

Inherited disorders of the cholesterol metabolism are, on the one hand, characterized by a dysfunction of the protein that should take up the extremely cholesterol-rich LDL particles from the blood (the LDL receptor), caused by a gene defect. The discovery of the association between defects in the LDL receptor gene and the consequent disorder, familial hypercholesterolemia, by Brown and Goldstein in Dallas, was awarded with the Nobel Prize for Medicine in 1985. On the other hand, the protein that, as a part of the cholesterol-containing LDL particle, should bind to the LDL receptor (the apolipoprotein B-100), can be defective, too. This disorder, described for the first time by research groups in Dallas and San Francisco in 1989, is referred to as familial-defective apolipoprotein B-100 (Figures 3a, 3b). In both disorders, cholesterol-rich LDL particles accumulate in the blood due to an insufficient cellular uptake. Accumulation of these particles in the blood (hypercholesterolemia) leads to an accelerated development of atherosclerosis. A complete dysfunction of the LDL receptor causes severe hypercholesterolemia (LDL cholesterol concentrations in the blood increased by 6-10 fold) that, if untreated, often provokes a heart attack already in childhood. Fortunately, this (homozygous) form of familial hypercholesterolemia where the child inherits a defective gene both from the father and the mother is very rare (approximately 1:1'000000).

However, even only one defective gene (inherited either from the father or the mother; heterozygous form) leads to a partly reduced functioning of the LDL receptor. This results in a marked increase (2-3 fold) in LDL cholesterol. Typically, without treatment affected individuals develop atherosclerosis unnoticed that can consequently manifest itself as a sudden heart attack already in early adulthood.

Familial-defective apolipoprotein B-100 – a frequent disorder of the cholesterol metabolism in the northern and western parts of Switzerland

In the gene encoding the LDL receptor more than 370 different defects have been discovered worldwide. In the gene encoding the apolipoprotein B-100 only three different defects that reduce the functioning have been identified so far.

Our own studies at the Department of Research, University Hospital Basel, demonstrated that one of these three defects in the apolipoprotein B-100 gene (amino acid substitution at position 3'500: arginine by glutamine) has, surprisingly, a significantly higher prevalence in Switzerland than in other countries. Especially in the northern and western parts of Switzerland this apolipoprotein B-100 deficiency has the highest prevalence worldwide. According to our studies familial-defective apolipoprotein B-100 affects approximately one in 200 Swiss, and thus, is more frequent than the estimated number of all defects of the LDL receptor gene taken together. Approximately one in 500 Swiss is affected by familial hypercholesterolemia, if the observations of Brown and Goldstein are applied to Switzerland.

The contribution of molecular genetics to the investigation of disorders of the cholesterol metabolism

The presence of one of the familial forms of hypercholesterolemia (familial hypercholesterolemia, familial-defective apolipoprotein B-100) can, in principle, be diagnosed on the basis of the increase in cholesterol, the mode of inheritance, and the presence of tendon xanthomata. Without molecular genetic tests, however, both disorders cannot be distinguished definitely. As far as younger individuals or affected members of small families are concerned, the clinical diagnosis of one of the familial forms,

especially in cases with only moderately elevated cholesterol levels in the blood, is also rendered difficult.

By the experience we gained in Basel over the last years, the direct confirmation or exclusion of the respective defect by molecular genetic methods is, therefore, often helpful. Moreover, we are able to classify affected individuals according to the more than 370 subgroups of familial hypercholesterolemia or to the three subtypes of familial-defective apolipoprotein B-100 known to date. In the near future, confirmation of one of these subtypes in a patient will probably also allow prognoses or have therapeutic consequences. We have already been able to demonstrate differences between familial-defective apolipoprotein B-100, highly frequent in Switzerland, and familial hypercholesterolemia: individuals affected by familial-defective apolipoprotein B-100 have, on the average, lower plasma cholesterol levels than individuals affected by familial hypercholesterolemia. In addition, with regard to familial-defective apolipoprotein B-100 the association between elevated cholesterol levels and age is more pronounced compared to familial hypercholesterolemia.

Our results published in 1995 were confirmed by a Dutch-Canadian research group in 19... Individuals affected by familial-defective apolipoprotein B-100 might also have a slightly lower risk to suffer from premature atherosclerosis than individuals with familial hypercholesterolemia. The conclusive answer to this question will be particularly important for the inhabitants of our region due to the relative frequency of this disorder.

Likewise, more significant differences are emerging between the various subtypes of familial hypercholesterolemia (defects of the LDL receptor) not only with regard to their regional distribution, but also to the severity of the disorder of the lipid metabolism. Due to the small number of cases in the respective subgroups we aim at a worldwide co-operation. Within the framework of an internationally co-ordinated project that involves scientists from more than 30 countries, this issue is being debated at present.

All first-degree relatives (mother, father, sisters, brothers, daughters, sons) of an individual affected by one of the familial forms of hypercholesterolemia have a calculated 50% risk to be equally affected by this disorder. Today, it is, therefore, regarded as a duty of the medical profession to identify and treat in time high-risk family members.

Approximately three years ago, in the context of a project supported by the World Health Organization WHO (MED PED: Make Early Diagnoses – Prevent Early Deaths) we launched, in Basel, a center at the Department of Research and the Division of Endocrinology, Diabetology, and Clinical Nutrition (Professor U. Keller) with the aim to make - for the whole of Switzerland - relatives of patients affected by familial forms of hypercholesterolemia aware of this potential risk, to identify affected family members and to refer them to their general practitioners for treatment. In subsequent years more centers followed at the University Hospitals of Lausanne, Geneva and Zurich. All centers offer counselling and cholesterol determinations; for the whole of Switzerland the respective molecular genetic tests are carried out in Basel where the data collection is centralized. As the data of, in most cases, healthy individuals is highly sensitive, great care is taken with regard to data protection. To this end we introduced some innovations such as the development of specialized software.

Regulation of the genes involved in the uptake and synthesis of cholesterol

Some years ago it could already be demonstrated that defects in the LDL receptor gene and the apolipoprotein B-100 gene lead to disorders of the cholesterol metabolism. Besides the effects of defects in these genes, two further genes - defects in humans are unknown - involved in the cholesterol metabolism recently described by Brown and Goldstein are of increasing interest. These genes, the sterol-regulatory-element-binding proteins (SREBP)-1 and SREBP-2 play key roles in the cholesterol homeostasis of the cell. They regulate the uptake of cholesterol from the blood and, at the same time, the intracellular cholesterol synthesis. Cholesterol is essential to the cell. Therefore it hardly comes as a surprise that the maintenance of the cellular cholesterol content is tightly regulated. The cell increases its cholesterol content by increasing the amount of LDL receptors that go to the cell surface, bind the cholesterol-rich LDL particles in the blood, and transfer them into the cell. However, the cholesterol synthesis within the cell itself is enhanced by increasing the production of proteins (so-called enzymes) involved in this production process, e.g. the hydroxymethyl-glutaryl-co-enzyme A (HMG-CoA) synthase, the HMG-CoA reductase, the farnesyl-pyrophosphate (PP) synthase and the squalene synthase (Figures 4a, 4b). The cholesterol content of the cell is regulated by the

transcription factors SREBP-1 and SREBP-2. A transcription factor is an endogenous protein that controls the activity of other genes by binding to a specific site of those genes, the promoter. If the cholesterol content of the cell is too low, mature forms of the transcription factors SREBP-1 and SREBP-2 are released. They enter the nucleus and bind to the promoters of the LDL receptor gene and of the genes responsible for the intracellular cholesterol synthesis. All these genes are activated simultaneously by the two transcription factors. Thus, on the one hand, more LDL receptors are produced, i.e. more cholesterol is taken up by the cell. On the other hand, more enzymes involved in the intracellular cholesterol synthesis are produced. As a consequence of these two mechanisms the cholesterol content of the cell increases, which, in turn, reduces the production of the transcription factors SREBP-1 and SREBP-2. Thus, a sophisticated feedback mechanism ensures the maintenance of a balanced cellular cholesterol content. Both SREBP-1 and SREBP-2 regulate the activity of the genes involved in this process. After identification of the structure of the SREBP-2 gene, which is probably more important for the regulation of cholesterol, and of the structures of the promoters of SREBP-1a, SREBP-1c, and SREBP-2 I successfully completed at the Department of Brown and Goldstein, we will now, as a further step, continue to investigate the functioning and regulation of these genes in our research group in Basel that is still in its initial stages. We are hoping that the investigation of these genes will provide further insights into the mechanisms regulating the cholesterol content of the cell and help us to understand why an increase in the LDL cholesterol content of the blood drastically accelerates the development of atherosclerosis.